REMARKS

Claims 1-24 are pending in the present application. Favorable reconsideration is respectfully requested.

The rejection of the claims under 35 U.S.C. §112, first paragraph (written description), is respectfully traversed.

The presently claimed invention has been amended to define the microorganism as belonging to the genus *Enterobacter*, the genus *Klebsiella*, the genus *Serratia*, the genus *Pantoea*, the genus *Erwinia*, the genus *Escherichia*, the genus *Corynebacterium*, the genus *Alicyclobacillus*, or the genus *Saccharomyces*. In view of this amendment, which finds support at page 12, lines 8-14, Applicants submit that the claimed invention meets the written description standard under 35 U.S.C. §112, first paragraph. MPEP § 2163.02 states:

> An objective standard for determining compliance with the written description requirement is, "does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed." *In re Gostelli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989).

In view of the description at page 12, lines 8-14 and the extensive examples proffered in the present application, Applicants submit that the specification provides an adequate description to allow the skilled artisan to recognize what has been invented and what is claimed is adequately described in the specification within the meaning of 35 U.S.C. § 112, first paragraph. More particularly, the present application provides a detailed description of a method for screening a microorganism that can metabolize a carbon source in a liquid medium containing L-glutamic acid at a saturation concentration and the carbon source at a specific pH. See page 8, line 27 to page 12, line 7 of the present specification. Therefore,

one skilled in the art can readily appreciate that a microorganism belonging to the claimed genera can be obtained by the screening method described in the present application.

Accordingly, withdrawal of this ground of rejection is requested.

The rejection of the claims under 35 U.S.C. §112, first paragraph (enablement), is respectfully traversed.

As stated above, the present application provides a detailed description of a method for screening a microorganism that can metabolize a carbon source in a liquid medium containing L-glutamic acid at a saturation concentration and the carbon source at a specific pH. See page 8, line 27 to page 12, line 7 of the present specification. Therefore, even in the case where a microorganism is one other than *Enterobacter agglomerans* (i.e. members of the additional claimed genera of Claim 1), one skilled in the art can understand that such a microorganism can be obtained by the screening method described in the present application.

As evidenced by the publications submitted with the response filed on July 24, 2003 (copy refiled on September 25, 2003), microorganisms other than Enterobacter *agglomerans* also have similar pathways and enzymes related to L-glutamic acid biosynthesis. Moreover, the genera of microorganisms in present Claim 1 are well known as L-glutamic acid-producing microorganisms. As evidenced by the **enclosed copy** of pages 178, 228, and 229 of "Bergey's Manual of Determinative Bacteriology, Ninth Edition (1994)," (labeled as the Reference 1) the genus *Klebsiella* and the genus *Serratia* are closely related to the genus *Enterobacter*. For example, in Table 5.12 on page 228, the genus *Klebsiella* and the genus *Serratia* are described as related genera to the genus *Enterobacter*. Similarly, the genus *Pantoea* and the genus *Erwinia* are also known to be related genera to the genus *Enterobacter*.

Further, the microorganisms belonging to the genus *Serratia*, the genus *Erwinia*, or the genus *Enterobacter* can be grown at a low pH. To demonstrate that the microorganisms can be grown, not only at pH 4.5 as evidenced by the Examples of the present specification, at pH below 4.5 Applicants **submit herewith** "the Reference 2."

Moreover, to further evidence the enablement of the present invention, Applicants **submit herewith** a Declaration executed by Yoshihiko Hara ("the Hara Declaration"). In the Hara Declaration, an experiment is reported in which strains having improved resistance to L-glutamic acid at a high concentration in a low pH environment are screened. In the attached sheets to the Hara Declaration, it is demonstrated that most of strains (named G1 to G35, G106, and S1 to S44, mutants of *Enterobacter agglomerans* SC17sucA/RSFCPG+pSTVCB) consumed more saccharide (glucose or sucrose) and grew faster than the control strains named "P" (*Enterobacter agglomerans* SC17sucA/RSFCPG+pSTVCB) at pH 4.7. Of all mutant *Enterobacter agglomerans* SC17sucA/RSFCPG+pSTVCB strains, G106 (AJ13601) strain exhibited excellent growth at the low pH and the same L-glutamic acid producing ability as that of the control strains at neutral pH. Strains G29 and G35 also provide similar results to those of G106 strain and, therefore, also have the claimed abilities (i.e., an ability to metabolize a carbon source at a specific pH in a liquid medium containing the carbon source and L-glutamic acid at a saturation concentration, and has the ability to accumulate in the liquid medium L-glutamic acid in an amount exceeding the amount corresponding to the saturation concentration at the specific pH).

MPEP § 2164.01 states:

> The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation.

In view of the foregoing, as well as the Examples of the present application, Applicants submit that the skilled artisan would be able to make and use the invention as claimed. More specifically, in view of the foregoing and the specification as filed, it would be well within the purview of the skilled artisan to make and identify microorganisms within the scope of the claimed invention. As such, Applicants request withdrawal of the rejection of the claims under 35 U.S.C. §112, first paragraph.

The rejection of Claims 1-10, 14, and 17-24 under 35 U.S.C. §102(e) over Moriya et al is traversed.

In the outstanding Office Action the Examiner asserts that Moriya et al disclose strains that have the presently claimed properties as, as such, anticipate the claimed invention. Applicants disagree with this assertion. More specifically, the undersigned has been informed that "from the inventors' knowledge, the strains ATCC12287/RSFCPG, AJ13355/RSFCPG, AJ13355/pMWCB, AJ13355/pSTVG, ATCC14460/RSFCPG, AJ13356, and AJ13356/RSFCPG described in Moriya are not the strains which have the ability of metabolizing a carbon source at a specific pH in a liquid medium containing L-glutamic acid at a saturation concentration and the carbon source, and which have ability to accumulate L-glutamic acid in an amount exceeding the amount corresponding to the saturation concentration in the liquid medium at the pH."

Moreover, transformation with RSFCPG and pSTVCB as disclosed in Moriya et al does not result in the claimed properties. To evidence this, Applicants **submit herewith** an Appendix in which *Enterobacter agglomeranse* strain SC17 was transformed with RSFCPG and pSTVCB and subsequently cultured, wherein the culture pH (4.7) was controlled by introducing ammonia gas into the medium. As the initially added glucose was depleted, 600

g/L of glucose was continuously added. The OD at 562 nm and L-glutamic acid accumulation of the culture were measured (see Section 2 "Results"). As shown in the Appendix, L-Glutamic acid production plateaued and crystal precipitation was not observed. Accordingly, transformation of *E. agglomeranse* strain SC17 with RSFCPG and pSTVCB fails to impart the claimed properties upon the microorganism disclosed by Moriya et al.

Based on the foregoing, Applicants submit that Moriya et al fails to anticipate the claimed invention and, therefore, this ground of rejection should be withdrawn.

Applicants respectfully request that the obviousness-type double patenting rejection of Claims 3-5, 7-10, 12-15, and 22 over Claims 1-13 of US 6,720,010 be held in abeyance until an indication of allowable subject matter in the present application. If necessary, a terminal disclaimer will be filed at that time. Until such a time, Applicants make no statement with respect to the propriety of this ground of rejection.

Applicants submit that the present application is in condition for allowance. Early notification to this effect is respectfully requested.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Stephen G. Baxter, Ph.D.
Attorney of Record
Registration No. 32,884

Vincent K. Shier, Ph.D.
Registration No. 50,552

Customer Number
22850

Tel: (703) 413-3000
Fax: (703) 413-2220
(OSMMN 08/03)
SGB/VKS

The Reference 1

Genus Edwardsiella

Small, straight rods, about 1 μm in diameter × 2–3 μm, Gram negative. Motile by peritrichous flagella (*E. ictaluri* is motile at 25°C but not at 37°C). **Facultatively anaerobic** and chemoorganotrophic, having **both a respiratory and a fermentative type of metabolism.** Optimal temperature is 37°C, except for *E. ictaluri,* which prefers a lower temperature. D-Glucose and some other carbohydrates are catabolized with the production of acid and often gas, but they are **inactive compared to most genera in** *Enterobacteriaceae.* **Oxidase negative, catalase positive, and Voges-Proskauer and Simmons citrate negative. Lysine decarboxylase and usually ornithine decarboxylase positive.** Reduce nitrates. Carbohydrates fermented by all species are maltose and D-mannose. Most frequently occur in the intestine of cold-blooded animals and their environment, particularly fresh water, but also occur in warm-blooded animals and humans. Pathogenic for eels, catfish, and other animals; they are a rare opportunistic pathogen for humans.

Type species: *Edwardsiella tarda.*

Edwardsiella is biochemically somewhat similar to *Escherichia coli, Shigella,* and *Salmonella,* but it is easily differentiated on the basis of a complete set of biochemical test results (Table 5.8).

Differentiation of the species of the genus **Edwardsiella:** Differential characteristics of *Edwardsiella* species are given in Table 5.9. Additional biochemical results are shown in Table 5.2.

Genus Enterobacter

Straight rods, 0.6–1.0 μm wide × 1.2–3.0 μm long, Gram negative. **Motile by peritrichous flagella (except** *E. asburiae).* **Facultatively anaerobic** and chemoorganotrophic, having **both a respiratory and a fermentative type of metabolism.** Optimal temperature is 30–37°C. D-Glucose and other carbohydrates are catabolized with the production of acid and gas. Indole negative. Most strains are **Voges-Proskauer positive and Simmons citrate positive.** Methyl red reaction varies. **Lysine negative** (except *E. gergoviae*) and **ornithine positive** (except *E. agglomerans*). **Malonate is usually utilized, and gelatin is slowly liquified** (3–14 days) by most strains. **H_2S, deoxyribonuclease, and lipase are not produced.** Carbohydrates fermented by all or most strains include L-arabinose, cellobiose, maltose, D-mannitol, D-mannose, salicin, and trehalose. Widely distributed in nature, occurring in fresh water, soil, sewage, plants, vegetables, and animal and human feces. Several species, most notably *E. cloacae, E. sakazakii, E. aerogenes, E. agglomerans,* and *E. gergoviae,* are opportunistic pathogens, causing burn, wound, and urinary tract infections and occasionally septicemia and meningitis.

Type species: *Enterobacter cloacae.*

Characteristics that distinguish *Enterobacter* species from klebsiellae are given in Tables 5.10 and 5.11. Table 5.12 lists characteristics that can be used to differentiate *Enterobacter* from *Klebsiella, Hafnia,* and *Serratia.*

Differentiation of the species of the genus **Enterobacter:** See Table 5.13.

Editorial note: The species listed below were not included in *Bergey's Manual of Systematic Bacteriology* or were briefly mentioned under "Other organisms belonging to the genus *Enterobacter.*" *Enterobacter amnigenus* was created by Izard et al. in 1981 (Int. J. Syst. Bacteriol. *31:* 35–42); *Enterobacter asburiae* was created by Brenner et al. in 1986 (J. Clin. Microbiol. *23:* 1114–1120; Int. J. Syst. Bacteriol. *38:* 220–222, 1988); *Enterobacter cancerogenus* was transferred to *Enterobacter* from the genus *Erwinia* by Dickey and Zumoff in 1988 (Int. J. Syst. Bacteriol. *38:* 371–374); *Enterobacter dissolvens* was transferred to *Enterobacter* from the genus *Erwinia* by Brenner et al. in 1986 (J. Clin. Microbiol. *23:* 1114–1120; Int. J. Syst. Bacteriol. *38:* 220–222, 1988); *Enterobacter hormaechei* was created by O'Hara et al. in 1989 (J. Clin. Microbiol. *27:* 2046–2049; Int. J. Syst. Bacteriol. *40:* 105–106, 1990); *Enterobacter intermedium* was created by Izard et al. in 1980 (Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C: *1:* 51–60; Int. J. Syst. Bacteriol. *30:* 601, 1980). The specific epithet *"intermedium"* did not agree in gender with the genus name and therefore has been changed to *intermedius* (von Graevenitz, Int. J. Syst. Bacteriol. *40:* 211, 1990). *Enterobacter nimipressuralis* was transferred to *Enterobacter* from the genus *Erwinia* by Brenner et al. in 1986 (J. Clin. Microbiol. *23:* 1114–1120; Int. J. Syst. Bacteriol. *38:* 220–222, 1988); and *Enterobacter taylorae* was created by Farmer et al. in 1985 (J. Clin. Microbiol. *21:* 77–81; Int. J. Syst. Bacteriol. *35:* 223–225, 1985). *Enterobacter agglomerans* is a senior subjective synonym of *Erwinia herbicola* and *Erwinia milletiae* (Beji et al., Int. J. Syst.

Table 5.12 Differentiation of *Enterobacter* and related genera

Test	*Enterobacter*	*Klebsiella*	*Hafnia*	*Serratia*
Motility	+[a]	–	[+]	+
Ornithine decarboxylase	+[b]	+	–	+[d]
Arginine dihydrolase	d	–	–	–
Deoxyribonuclease	+	+	–	+
Citrate utilization	+	+[e]	–	+
Susceptible to *Hafnia* phage	–	–	+	–

Symbols: see Table 5.2 on p. 222.

[a] *E. asburiae* and almost one-half of *E. hormaechei* strains are nonmotile.

[b] *E. agglomerans* is negative.

[c] *S. odorifera* biogroup 2, *S. plymuthica*, and *S. rubidaea* are negative.

[d] *S. grimesii* is positive.

[e] *K. pneumoniae* subsp. *rhinoscleromatis* and some strains of *K. pneumoniae* subsp. *ozaenae* are negative.

Table 5.13 Biochemical differentiation of *Enterobacter* species

Test	*E. aerogenes*	*E. agglomerans*	*E. amnigenus* biogroup 1	*E. amnigenus* biogroup 2	*E. asburiae*	*E. cloacae*	*E. dissolvens*
Methyl red	–	d	–	d	+	–	–
Voges-Proskauer	+	d	+	+	+	+	+
Urea hydrolysis	–	[–]	–	–	d	d	+
Lysine decarboxylase	+	+	+	+	+	+	+
Arginine dihydrolase	–	–	–	d	[–]	+	+
Ornithine decarboxylase	+	+	d	+	+	+	+
Motility	+	[+]	+	+	–	+	+
Gelatin hydrolysis 22°C	–	–	–	–	–	–	–
KCN, growth	+	d	+	+	+	+	+
Malonate utilization	+	d	+	+	+	[+]	+
Acid production from:							
D-Adonitol	+	+	+	+	+	[+]	+
Dulcitol	–	[–]	–	–	–	[–]	–
Glycerol	+	d	+	+	[+]	d	+
myo-Inositol	+	[–]	–	–	–	[–]	d
Melibiose	+	d	+	+	+	+	+
α-CH_2-D-glucoside	+	–	d	+	+	[+]	+
Raffinose	+	d	+	+	d	+	+
L-Rhamnose	+	[+]	+	+	–	+	+
D-Sorbitol	+	d	+	+	+	+	+
Sucrose	+	[+]	+	–	+	+	+
Yellow pigment 25°C	–	[+]	–	–	–	–	–

Table 5.13 *(continued)*

Test	*E. gergoviae*	*E. hormaechei*	*E. intermedius*	*E. nimipressuralis*	*E. sakazakii*	*E. taylorae*
Methyl red	–	d	+	–	–	–
Voges-Proskauer	+	+	+	+	+	+
Urea hydrolysis	+	[+]	–	–	–	–
Lysine decarboxylase	+	+	+	+	+	+
Arginine dihydrolase	–	[+]	–	+	+	+
Ornithine decarboxylase	+	+	+	+	+	+
Motility	+	d	+	+	+	+
Gelatin hydrolysis 22°C	–	–	–	–	–	–
KCN, growth	–	+	d	+	+	+
Malonate utilization	+	+	+	+	+	+
Acid production from:						
D-Adonitol	–	–	–	–	–	–
Dulcitol	–	[+]	+	–	–	–
Glycerol	+	+	+	+	+	+
myo-Inositol	–	–	–	–	[+]	–
Melibiose	+	+	+	+	+	+
α-CH_2-D-glucoside	–	[+]	+	+	+	–
Raffinose	+	+	+	+	+	+
L-Rhamnose	+	+	+	+	+	+
D-Sorbitol	–	–	–	–	–	–
Sucrose	+	+	d	–	+	–
Yellow pigment 25°C	–	–	–	–	–	–

Symbols: see Table 5.2 on p. 222.

Important Notes for Users of This Manual

Unless otherwise indicated in footnotes to tables, the meanings of symbols are as follows:

- + 90% or more of strains are positive
- – 90% or more of strains are negative
- d 11-89% of strains are positive
- v strain instability (not equivalent to "d")
- D Different reactions in different taxa (species of a genus or genera of a family)

All other symbols are defined in footnotes to tables.

The Reference 2

The culture of the bacterium of the present invention in the medium whose pH is below 4.5

Enterobacter agglomerans AJ13355, Serratia rubefaciens AJ2712 and Erwinia herbicola AJ2169 were inoculated into LB medium, respectively and cultured over night.

Then, each of 80 μl of the cultured medium was inoculated into 4ml of the culture medium having the following composition in test tubes, respectively.

[Composition of culture medium]

Glucose: 50g/L; $MgSO_4 \cdot 7H_2O$: 0.625g/L; $(NH_4)_2SO_4$: 8.33g/L ; Glutamic acid: 16.7g/L; $CaCl_2$: 208mg/L; KH_2PO_4: 1.67g/L; YE: 1.67g/L; NaCl: 0.42g/L; $FeSO_4 \cdot 7H_2O$: 16.7mg/L; pH was adjusted to pH 4.3 or pH 4.1 by adding ammonia gas.

Then, we cultivated the above bacteria and observed the growth of the bacteria with time by measuring the cell density at 570nm with a spectrophotometer. The results of these experiments are shown below.

	(a)	(b)	(c)	(d)
Time (h)				
0.0	0.107	0.130	0.095	0.113
1.5	0.158	0.135	0.09	0.135
2.7	0.320	0.175	0.123	0.19
3.8	0.500	0.213	0.19	0.257

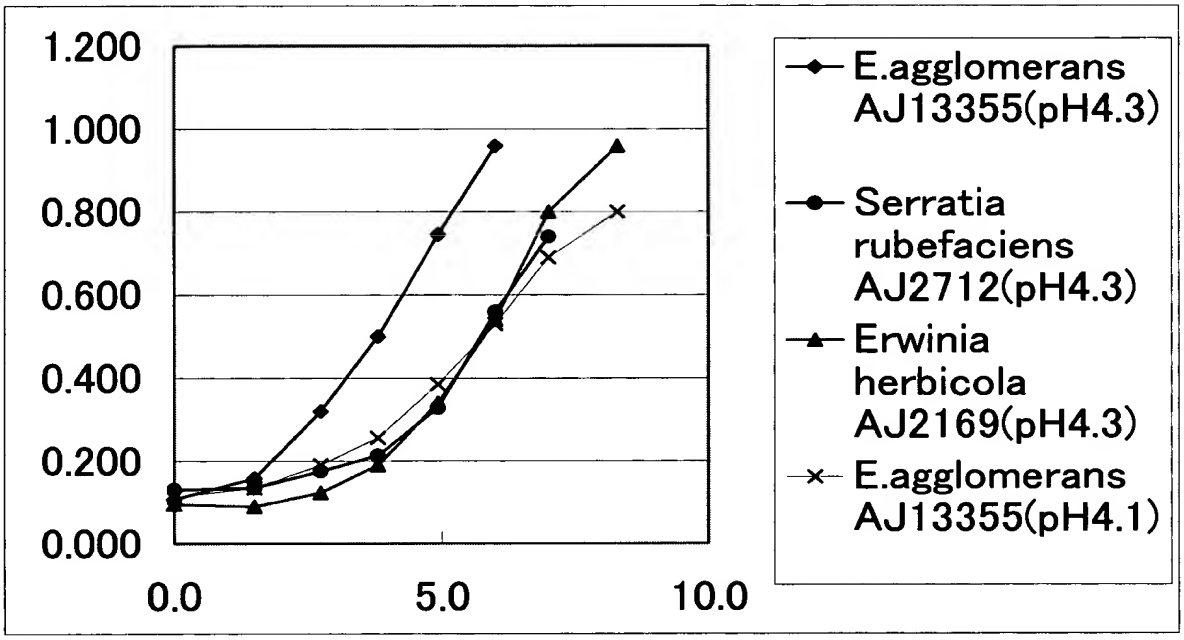
4.9	0.745	0.329	0.34	0.385
6.0	0.960	0.560	0.54	0.53
7.0		0.740	0.8	0.69
8.3			0.96	0.8

(a): Enterobacter agglomerans AJ13355 (pH 4.3)

(b): Serratia rubefaciens AJ2712 (pH 4.3)

(c): Erwinia herbicola AJ2169 (pH 4.3)

(d): Enterobacter agglomerans AJ13355 (pH 4.1)



As shown in the above results, we found that the above bacteria of the present invention can proliferate in a medium even when the pH of the medium is below 4.5.